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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

We are currently investigating a putative tumor suppressor gene (TSG) located at chromosomal band 8p22 that is involved in breast cancer. We have proposed to apply the genetic suppressor element (GSE) approach to the identification of this TSG. Briefly, a library of short gene fragments will be introduced into a cell line which demonstrates suppression of clonogenicity in soft agar with the transfer of chromosome 8. Presumably, the 8p22 TSG is responsible for the suppression of clonogenicity, and the introduction of an "active" GSE from the library into the suppressed cells should inhibit the 8p22 TSG contained in the hybrid cells and allow reversion back to the parental phenotype, the ability to grow in soft agar. Any clones that form will be isolated and further evaluated, as a candidate for the TSG. Currently, a complete list of ESTs have been identified in the 8p22 interval using various methods. A GSE library consisting of these ESTs has been constructed and evaluation of the library is currently underway. All preliminary optimization experiments for retroviral library delivery have been completed. The majority of candidate ESTs have been partially characterized aand full length cDNAs obtained. Additionally, one candidate has been identified as a likely potential downstream target of the 8p22 TSG from differential gene expression studies, this gene is currently being analyzed individually in our MB231 hybrid/parental assay system to evaluate its functional significance in breast cancer cells

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Introduction

Breast cancer is generally characterized as a number of pathologically distinct entities that constitute the progression of lesions from hyperplasia, to carcinoma in situ, to invasive carcinoma, finally resulting in disease metastasis (1). Tumor suppressor genes (TSG) have been known to play an important role in the initiation and progression of human cancers, and there exists a large body of data that suggests the existence of a TSG on the short arm of chromosome 8 (8p). 8p is of particular interest in breast cancer because it is a site of frequent allelic loss in breast tumors, and it has been shown that 8p loss is one of the most common events in breast cancer (2). However, 8p loss is seen in a wide range of epithelial carcinomas (3-7), and is thought to be non tissue-specific. Several regions of 8p have been identified by LOH as putative TSG sites, including 8p22, where a homozygous deletion was reported in prostate cancer (8). Since homozygous deletions are regarded as the most sensitive indicator of TSG presence, we have chosen to concentrate our efforts in this region. Furthermore, others in our lab have shown functional evidence that chromosome 8 harbors tumor suppressor activity through monochromosome 8 transfer into cell lines derived from breast tumors, resulting in a decrease in tumorigenicity and clonogenicity in soft agar (unpublished). These matched cell line pairs offer an in vitro phenotype that is easily scorable and provides a model assay system. Using this model, we have proposed to use a functional negative selection approach to identify a tumor suppressor gene on 8p that is involved in breast carcinoma. In brief, we have proposed to construct a library of genetic suppressor elements (GSE), which consists of short gene fragments which encode inhibitory sense or antisense RNA (9-10), and introduce this library into our suppressed chromosome 8 containing breast cancer cell lines. Any active GSEs will inhibit the function of the putative TSG on 8p in these suppressed lines and as a result, "unsuppress" the cell clone, thus allowing colony formation in soft agar. Isolation, expansion, identification, and verification of the clones will follow. Finally, any genes identified will be characterized, and evaluated as a candidate TSG. While the overall progress of our study has been considerable, and much of the statement of work has been accomplished, we have deviated from the order in which we have performed the proposed tasks, for reasons explained below. Our progress for the funding period from September 2000-August 2001 is outlined as follows.

Objective 1: Creation of GSE library (Task 1)

We had originally proposed to prepare a genetic suppressor element (GSE) library using bacterial clones from a physical map of 8p22 that we have constructed in our laboratory. However, as mentioned in the previous annual summary, since our original submission, we have decided to construct a more specific library of decreased complexity using ESTs that have been mapped to and around our interval.

We previously reported the identification of several ESTs that map to our region. During the past funding period, we have accomplished a complete list of all ESTs that lie in and around the target interval, due largely in part to the recent completion and release of the human genome sequence. We identified of a total of 8 genomic clones sequenced by the Human Genome Project as falling within the target 8p22 interval by comparing alignment with sequence from our previously assembled bacterial contig. The bacterial clones identified are as follows: AI126483, AC008005, AC018437, AC019292, AC010785,

AC022526, AC023396, AC010656. These genomic clones were searched against dbEST to identify additional transcripts. A complete list of all identified ESTs is given below.

Unigene ID	Comments
Hs. 223816	N33 (TIGR)
Hs. 4997	Hypothetical protein FLJ10482
Hs.110796	SAR1
Hs.127056	N/A
Hs.133897	N/A
Hs.145689	N/A
Hs.147635	N/A
Hs.1708	Chaperonin cont. TCP1, subunit 3, (gamma)
Hs.170980	N/A
Hs.201818	N/A
Hs.236366	Highly similar to N33 protein
Hs.252691	Found only in brain
Hs.254843	N/A
Hs.255425	Homo sapiens cDNA: FLJ22496 fis, clone HRC11236
Hs.266796	R80652
Hs.270251	R26196
Hs.283526	N/A
Hs.288363	RETIRED...N33
Hs.49	MSR1
Hs.57764	Protein phosphatase 1A (2C)
Hs.71119	N33
Hs.104143	Clathrin, light polypeptide (Lca)
Hs.296330	N/A

Over the past funding period we have completed construction of the GSE library from the identified cDNAs. Validation of the integrity and representation of library is currently underway. Since the all of the ESTs identified are commercially available, we slightly redesigned our approach for library construction. Consensus PCR primers suitable for the amplification of cDNA inserts from any plasmid vector in the human subset of the Unigene collection are commercially available (Research Genetics). We modified these commercial primers by introducing one of two different restriction enzyme sites (EcoRI or BamHI) into the reverse and forward primers, to be able to directionally clone PCR products in sense and anti–sense orientations. We PCR–amplified the cDNA inserts individually in the 96–well format. PCR–amplified products were then pooled and cloned as a library into the retroviral vector pBABEpuro. Once the library integrity is verified, we will proceed with introduction of the library into the target breast cancer cell lines.

As described in the previous summary report, we have expanded our investigation to include possible targets of the 8p22 TSG that have resulted from differential gene expression studies. One gene that has emerged as a strong potential downstream target of the 8p22 TSG, PMEPA1, shows a significant increase in expression level in the

suppressed chromosome 8-containing breast and colon cancer cell lines, when compared to the malignant parental lines. This gene was originally identified as an androgen regulated gene in prostate cancer cells (11). During the past funding period, we have cloned this gene in sense and anti-sense orientations into the pBABEpuro vector to evaluate its functional significance in our cell line pairs.

Objective 2: Introduction of library into target cells (Task 1)
As outlined in the previous report, we have completed all preliminary studies to optimize the retroviral packaging system including transfection and infection protocols, and the soft agar cloning assay. Introduction of the GSE library into the target breast cancer cells is awaiting verification of the library integrity and representation of ESTs. The changes discussed above in construction of the library from the original proposal has slightly delayed its completion. However, since substantial progress has been made toward completion of task 2, the overall timeline will not be significantly altered.

During the most recent funding period, the sense and anti-sense constructs for PMEPA1 were transfected into the Phoenix A packaging cell line, followed by infection of the chromosome 8-containing MB231 cells, and the HT29 colon cancer cell line pair. Following retroviral infection, the target cells were expanded under puromycin selection for about 10 days. To assess the functional significance of this gene in vitro, the infected MB231 and HT29 cells were plated in soft agar. The assay is currently underway and the results will be available shortly.

Objective 3: Characterization of GSEs (Task 2)
We have already accomplished a significant part of Task 2 as outlined in the statement of work. We have already obtained the full-length cDNA sequence for the majority of the candidates from the TIGR and UNIGENE databases, and are attempting to accomplish this for all of them. In the event that our GSE library does not yield any active GSEs, we will be equipped to test each EST individually. In addition, all the candidates which are being included in our GSE library have been tested, and are periodically rechecked for match of identity or homology with any known sequences or previously identified transcripts.

As we have identified one very strong candidate for a downstream target of the putative 8p22 TSG, PMEPA1, we have expanded our studies to analyze this particular gene individually. This gene was previously cloned by another group, and thus we have the full-length cDNA at hand. Functional characterization is underway, as described above.

Training
During this past funding period, the departmental preliminary examination, a requirement for doctoral candidacy was successfully completed. This involved preparation of a hypothetical research proposal along with an oral defense of the proposed research. Although a requirement, this exercise provided valuable training for writing of research proposals, as well as oral presentation. Additional training has continued in the form of

particpation in regular journal clubs, seminars, lab meetings, and workshops. In addition, attending the national meetings for the American Society of Human Genetics and American Association of Cancer Research has been an invaluable training opportunity. Among the training benefits of attending national conferences has been experience in preparation and presentation of posters.

Key Accomplishments

- Completion of gene/EST identification within the 8p22 TSG interval.
- Completion of GSE library construction.
- Cloning PMEPA1 in sense and antisense orientations into the pBABEpuro retroviral vector.
- Cloning of a GFP-containing vector construct as a control to determine transfection and infection efficiencies.
- Successful completion of the departmental preliminary exam necessary for proceeding on to doctoral candidacy.
- Attending a national meeting of the American Society of Human Genetics.
- Attending and presenting poster in poster discussion session at American Association of Cancer Research annual meeting.

Reportable Outcomes

Banerjee K, Arvieva ZH, Spanknebel KA, Usha L, Sharma TT, Liang J, Gomes I, Westbrook CA. Differential gene expression in malignant breast and colon cancer cells and their suppressed counterparts. Am. J. Human Genetics 69 (suppl): 271, 2000.

Banerjee K, Arbieva ZH, Usha L, Le TT, Liang J, Gomes I, Westbrook CA. Identification of downstream targets of the putative tumor suppressor gene on 8p by differential gene expression analysis. Proceedings, American Association for Cancer Research 42: 428, 2001.

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